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Research Paper

Determination Concentrations of Vitamin D_3 in Suaeda maritima

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Abstract

In this research a simple and sensitive method was proposed for determination of vitamin D_3 in leaves of *Suaeda maritima* by HPLC after extraction. The variables affecting the chromatographic conditions and optimization of pH, flow rate, solvent ratios, and temperature were studied. A phosphate buffer solution with a pH of 3.0 and a flow rate of 1.0 ml min^{-1} at 25°C was chosen as optimal for peak resolution and better peak shape in a shorter run time. The relative standard deviation was less than 3%, and recovery was in the range of 74% to 85%.The method was successfully applied to determination of concentration vitamins D_3 in the halophytes samples.

Keywords: Halophytes; Vitamin D3; Solid Phase Extraction

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1. Introduction

Vitamins are essential organic compounds play a minor, but essential role to promote normal growth in humans and animals [1-2]. The vitamins are either fat-soluble (A, D, E, K) or water-soluble (B, C). The fat-soluble vitamins were extracted from the nutritional supplement samples without causing chemical change. Vitamin D is a group of fat-soluble has two major forms, ergocalciferol (D_2) and cholecalciferol (D_3) [3-5].

Herbs are an important part of a healthy balanced diet to supply needed minerals and vitamins [6]. Halophytes live in saline conditions of up to 200 mM NaCl and shows optimal growth under these conditions. They employ a variety of mechanisms to accommodate salinity, one of which is altering their energy metabolism. Moreover, they can store inorganic ions and have high osmotic potential to absorb water [7-8]. *S.maritima* is common species of the chenopodia family of herbal mangroves. Their native habitat is in the salt marshes of the northern hemisphere. They grow along high and low tide lines from April to October. A diet enriched with *S. maritima* can improve blood physiology and immunity. The growth rate of *S. maritima* decreases by July and increases after August. Most populations wither after September. The leaf of *S. maritima* has been used to treat hepatitis and shows antiviral properties. The young leaves are often mixed with other herbs to decrease their saline content [9-10].

Most samples are not suitable for direct introduction into analytical instruments. For this reason, the sample preparation procedure is an important step in an analytical study. Solid phase extraction (SPE) is used for preconcentration and separation of inorganic and organic species. SPE enhances the selectivity and sensitivity of a method by allowing discriminatory binding of an analyte to a solid support where it accumulates and subsequently elutes with a small volume of solvent. This technique has a higher enrichment factor, requires no emulsion, is safe for use with hazardous samples, has a minimal cost because it consumes little reagent, is environment friendly, flexible and easier to incorporate into automated analytical techniques. Preconcentration

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steps such as SPE are necessary for HPLC to remove interfering components [11-15]. The present study determined the amount of vitamin D_3 in the leaves of *S. maritima* using HPLC. The proposed method was used to determine vitamin D_3 of *S. maritima* by using HPLC.

2. Experimental

2.1 Chemicals and reagents

The analytical-reagent grade of vitamin (>99%) were purchased from Sigma Aldrich (Steinheim, Germany). The stock solutions (1000 ng L^{-1}) of each vitamines were prepared by dissolving each of them in methanol. The working solutions were prepared by appropriate dilution of the stock solutions with double distilled water. All of the standard solutions working standards were stored at 4 °C and brought to ambient temperature just prior to use. In throughout the experimental runs all the solvents, calibration and real samples were filtered through 0.22 µm nylon filter membranes (Varian, USA).

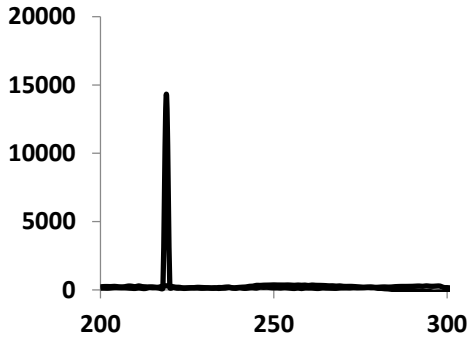

Figure 1.Wavelengthof vitamin D_3 (221 nm) .

2.2 Apparatus and software

HPLC (Shimadzu, LC20A System), vacuum degasser and system controller (SCL-10Avp) was used for testing. Chromatographic separation was conducted on a C18 (250mm×4.6mm,5µm) column . UV detection was performed at 200 to 300 nm with a spectral resolution of 1.0 nm and integration period of 0.4 s per spectrum (Figure 1). The mobile phase consisted of water and methanol (35:65, v/v). Prior to preparation of the mobile phase, water and methanol were degassed separately using a Millipore vacuum pump. The 0 mL min^{-1}. The pH was measured using a pH meter (Metrohm 827, Switzerland) combined with a glass electrode. Before use, the pH meter was calibrated against standard Merck buffers (pH = 4 and pH = 7). The sample (20 µL) was injected into the HPLC with a syringe (Hamilton). A 320R Hettich centrifuge (Germany) and a digital 10P ultrasonic bath (Sonorex; Germany) were used. The vitamin standards were of analytical-reagent grade from Sigma Aldrich (Germany).

2.3 Sample preparation

Standard stock solutions were prepared by dissolving each analyte in deionzed water with concentration of 500 µg mL^{-1}. Working standard solutions at different concentrations were prepared freshly by mixing the appropriate volumes of the stock solutions and diluting with deionized water.

The present study was carried out on samples of the halophytes *S.maritima* found in three regions of Hendijan in Khuzestan province of Iran. They were picked in early summer of 2013. The samples were dried after separating the leaves from the stalks and then rinsed with distilled water. The concentrations of vitamin D_3 were determined using an HPLC apparatus. The tests were repeated three times, since the samples of *S.maritima* contained many components that cause chromatographic interference with the vitamins.

3. Results

To obtain the best chromatographic conditions and shortest separation time were investigated, the influence of the analytical parameters in mobile phases with different pH values, and column oven temperatures. The aim of this study is to develop a simple, accurate and sensitive HPLC method for simultaneous determination of vitamin D_3 in halophytes samples.

3.1 Optimization of conditions

The variables affecting the chromatographic conditions and optimization of pH, flow rate, solvent ratios, and temperature were studied and optimized.

3.2 Effect of pH

Phosphate buffers of pH 3.0 to 6.0 were investigated to improve the resolution and peak symmetry. The pH level was found to be important to the separation process. It was found that higher pH (6.0) and lower pH (3.0) values increased the tailing of the peak and decreased the resolution. A pH of 3.0 was chosen as the optimum value for good resolution, better peak shape and a short run time.

3.3 Effect of mobile phase

Various ratios of solvents were tested. It was found in the mobile phase that the ratio of methanol and water affected the symmetry of the peak shapes. A mobile phase containing a water/methanol mixture phosphate buffer solution at pH = 3.0, a flow rate of 1.0 ml min^{-1} was used. An elution gradient was chosen that allowed complete analysis in less than 13 min..

3.4 Effect of temperature

The mobile phase was pumped at column oven temperatures of 20°C to 45°C. A temperature of 25°C was selected as optimum for the separation of these vitamins. The peak shapes and heights improved and the retention times decreased as the temperature increased.

3.5 Application of the proposed method to real sample

To evaluate performance of the proposed method, determination of vitamin D_3 in *S. maritima* sample were carried out under the optimized conditions that mentioned above. The results indicate that vitamin D_3 content for *S.maritima* are shown in Fig. 1. Concentration analysis of vitamins and accuracy data vitamins D_3 in real sample are shown in Table I and II.

Table I. Concentration analysis of vitamin D_3 by HPLC ($ngkg^{-1}$, n=3)

Vitamin(n=3)	Concentration($ngkg^{-1}$)	RSD(%)
D3	5	2.7
	100	3.0

Table II Accuracy data vitamin D_3 for spiked in real sample

Vitamin	*S. maritima*(ngkg_1)	S.maritima($ngkg^{-1}$)
D_3	29±0.35	17.8±0.4

4. Conclusion

The evaluation of the presence of vitamin D_3 in *S.maritima* can be used to determine the level of vitamins under different weather conditions and to determine which organs of the plant are most useful for preparation of extracts for use in humans. The proposed method examined the variables affecting chromatographic conditions and optimization of pH, flow rate, ratios of the solvents and temperatures. Phosphate buffer solution at a pH of 3.0 and a flow rate of 1.0 ml min^{-1} at 25°C was chosen as optimal for good resolution, better peak shape and short run time.

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